ORIGINAL ARTICLE VIROLOGY

Epidemiology of HIV-1 subtypes in an urban area of northern Italy

M. De Paschale[1], D. Cagnin[1], T. Cerulli[1], M. Mena[2], C. Magnani[2], P. Perini[2], T. Re[2], M. Villa[2], P. Viganò[2], C. Maltempo[3], M. T. Manco[1], C. Agrappi[1], P. Mirri[1], A. Gatti[1], C. Rescaldani[1] and P. Clerici[1]

1) *Microbiology Unit,* 2) *Department of Infectious Diseases, Hospital of Legnano and* 3) *SERT Magenta, ASL Provincia Milano 1, Milan, Italy*

Abstract

The distribution of the different subtypes of HIV varies from one region of the world to another. Subtype B is predominant in Europe and the USA, but there has been a gradual increase in non-B subtypes as a result of migration from regions where they are endemic, and this may have important implications for the control of HIV-1. The aim of this study was to assess the prevalence of HIV-1 subtypes in an urban area of northern Italy in the period 1997–2008. Forty-nine (12.2%; 95% CI, 9.00–15.40) of 401 patients investigated carried a non-B subtype, the prevalence of which was 7.7% (95% CI, 4.96–10.44) among native Italians and 55.3% (95% CI, 39.49–71.11) among non-Italians, 1.6% (95% CI, 0.00–3.81) among ex-intravenous drug addicts, 7.6% (95% CI, 1.21–13.99) among homosexual/bisexual men and 20.5% (95% CI, 14.83–26.17) among heterosexuals, 6.8% (95% CI, 3.37–10.23) among Italians infected as a result of sexual contacts in Italy, and 55.0% (95% CI, 33.20–76.80) among Italians infected abroad or by foreign partners. Overall prevalence increased from 2.9% (95% CI, 0.00–6.11) before 1993 to 23.0% (95% CI, 16.31–29.69) in the period 2001–2008. The results demonstrate that there has been an increase in non-B subtypes (especially sexually transmitted infections), particularly among patients infected abroad or by foreign partners.

Keywords: Epidemiology, heterosexual, intravenous drug user, men who have sex with men, type 1 human immunodeficiency virus subtypes
Original Submission: 2 April 2010; **Revised Submission:** 9 August 2010; **Accepted:** 15 September 2010
Editor: J.-M. Pawlotsky
Article published online: 27 September 2010
Clin Microbiol Infect 2011; **17:** 935–940
10.1111/j.1469-0691.2010.03382.x

Corresponding author: M. De Paschale, Microbiology Unit, Hospital of Legnano, Via Candiani 2, 20025 Legnano, Milan, Italy
E-mail: massimo.depaschale@ao-legnano.it

Introduction

Type 1 human immunodeficiency virus (HIV-1) is characterized by a wide range of genetic variability and, by means of phylogenetic analyses, has been classified into three distinct groups: M (major), O (outlier) and N (not M, not O). Group M is responsible for most of the infections in the world, and groups O and N for infections in limited regions of central Africa. Group M includes nine subtypes (AD, FH, J and K) [1] with genetic differences of 25–35% between them at the level of the *env* gene and 15% at the *gag* gene [2]. Analyses of the entire viral sequence have also led to the characterization of inter-subtype recombinants, which probably originated in individuals infected with two or more subtypes. The term circulating recombinant forms (CRFs) is used when an identical recombinant is isolated in at least three epidemiologically unrelated individuals [3].

The distribution of the subtypes and CRFs varies in different regions of the world [4,5]: subtype B predominates in North America, Western Europe and Oceania; subtype A in Eastern Europe and Central Asia; subtype C in South Africa, India and Ethiopia; subtype D in North Africa; and subtype G in West Africa. The other subtypes are mainly present (albeit in a minority) in Latin America and Eastern Europe (F), and Central Africa (F, H, J) [4]. CRFs are responsible for a large proportion of infections in some geographical regions, such as CRF01_AE in Southeast Asia and CRF02_AG in Central and West Africa [4].

There have been reports of associations between the different subtypes/recombinants and transmission routes. Subtype B is the most highly represented in Western countries, where HIV-1 initially manifested mainly in risk groups such as intravenous drug users (IVDUs) and men who have sex with men (MSM), whereas the other subtypes (particularly A and C) are more represented in developing countries, where transmission is primarily heterosexual [6]. Even within the same region, the subtypes may be segregated into different risk groups: for example, subtype B in IDVUs and CRF01_AE in heterosexuals has been reported in Thailand [7], and

subtype B in MSM and subtype C in heterosexuals in South Africa [8]. However, more important than a close link between a specific subtype and a route of transmission is determining the arrival of a subtype in a geographical area via a particular route of transmission, as subtype A spread among heterosexuals in Africa but among IVDUs in Eastern Europe. Changes in the route of transmission can also lead to epidemiological changes within the same population, as has been observed in Thailand where the original subtype B present in IVDUs has gradually been supplanted by CRF01_AE as a result of sexual transmission [9,10].

In Europe and the USA, where the predominant subtype B was historically introduced by IDVUs and MSMs, non-B subtypes and CRFs have become more frequent as a result of migration and sexual contacts between Europeans and people coming from regions in which non-B subtypes are endemic [11–16].

This has a number of implications because the different subtypes of HIV-1 may lead to differences in the progression to AIDS [17,18], although this is still controversial because the results of some studies have not been confirmed by others [19]. However, there do seem to be significant differences in the use of the CCR5 and CXCR4 co-receptors, and in the ability to induce syncytia [20]. In therapeutic terms, the different subtypes seem to have the same susceptibility to antiviral drugs [21], but some seem to produce mutations faster than others [22]. Subtype C seems to be naturally resistant to non-nucleoside reverse transcriptase inhibitors (NNRTIs) [23], whereas subtype G is less susceptible to protease inhibitors (PIs) *in vitro* [24].

The increasing circulation of new subtypes may therefore have major effects on controlling HIV-1, thus making public health surveillance increasingly important. The aim of this study was to assess the prevalence of the subtypes of HIV-1 (group M) in HIV-positive subjects in an urban area of northern Italy.

Materials and Methods

The study was based on samples taken from 401 HIV-1 positive subjects (278 men and 123 women, average age 40.7 years, range 14–70), which were received between 1997 and 2008 for the detection of resistance to antiretroviral drugs. At the time of collection, 159 patients (39.7%) were naïve and 242 (60.3%) had experienced treatment failure; 363 (90.5%) of the patients were Italian and 38 (9.5%) of foreign origin.

In order to search for resistance to antiretroviral drugs, HIV-RNA was extracted, reverse transcribed, amplified and sequenced using a commercial kit (TRUGENE HIV-1 Genotyping kit; Siemens, Tarrytown, NY, USA) in accordance with the manufacturer's instructions. The nucleotide sequence was sent by computer to the database of the American National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi) for subtyping.

The patients' medical records were consulted in order to record transmission routes, the probable geographical origin of the infection, and the date of the first detection of anti-HIV antibodies.

Fisher's exact test and the χ^2 test were used to analyse the data statistically.

Results

Three hundred and fifty-two samples (87.8% with a 95% confidence interval (CI) between 84.60 and 91.00) showed subtype B, and 49 (12.2%; 95% CI, 9.00–15.40) non-B subtypes, of which 18 (36.7%; 95% CI, 23.20–50.20) were CRFs. The differences between men and women were not statistically significant except in the case of CRF02_AG ($p < 0.01$) (Table 1).

The subtypes were divided on the basis of the patients' nationality. The prevalence of non-B subtypes was 7.7% (95% CI, 4.96–10.44) among native Italians and 55.3% (95% CI: 39.49–71.11) among non-Italians. The differences in the prevalence of the subtype among the patients of Italian and foreign origin were all statistically significant except for subtypes C and F (Table 1).

Non-B subtypes were found in the samples of 12.0% (95% CI, 2.99–21.01) of the patients aged <30 years and in 22.8% (95% CI, 11.91–33.69) of those aged more than 50 years (Table 2). The difference between the patients aged ≤50 and >50 years was statistically significant ($p < 0.05$).

The probable route of infection was available in 390 cases: in 124 (31.8%) the infection was due to the shared use of syringes among IVDUs; in 261 (69.9%) it was due to sexual contacts (195 heterosexuals, 60 MSM and six bisexuals); in three (0.8%) it was due to vertical transmission; in one (0.3%) it was due to blood transfusion; and in one (0.3%) it was due to tattooing. The prevalence of the HIV-1 subtypes in these patients is shown in Table 3. The difference between the ex-IVDUs and all of the subjects infected as a result of sexual contacts (heterosexual, MSM/bisexual) was statistically significant ($p < 0.01$).

All of the non-Italian patients were infected as a result of sexual contacts.

The date of the first positive HIV antibody test was available in 382 cases, and was used to estimate the assumed

TABLE 1. HIV-1 subtypes in patients, by gender and nationality

	HIV-1 positive patients						
	Gender			Nationality			
HIV-1 subtypes	Male	Female	p	Italian	Foreign	p	Total
B	247 (88.8%) 95% CI: 85.09–92.51	105 (85.4%) 95% CI: 79.16–91.64	NS	335 (92.3%) 95% CI: 89.56–95.04	17 (44.7%) 95% CI: 28.89–60.51	<0.01	352 (87.8%) 95% CI: 84.60–91.00
C	3 (1.1%) 95% CI: 0.00–2.33	1 (0.8%) 95% CI: 0.00–2.37	NS	3 (0.8%) 95% CI: 0.00–1.72	1 (2.6%) 95% CI: 0.00–7.66	NS	4 (1.0%) 95% CI: 0.03–1.97
F	18 (6.5%) 95% CI: 3.60–9.40	4 (3.3%) 95% CI: 0.14–6.46	NS	19 (5.2%) 95% CI: 2.92–7.48	3 (7.9%) 95% CI: 0.00–16.48	NS	22 (5.5%) 95% CI: 3.27–7.73
G	2 (0.7%) 95% CI: 0.00–1.68	3 (2.4%) 95% CI: 0.00–5.10	NS	2 (0.6%) 95% CI: 0.00–1.39	3 (7.9%) 95% CI: 0.00–16.48	<0.01	5 (1.2%) 95% CI: 0.13–2.27
CRF01_AE	2 (0.7%) 95% CI: 0.00–1.68	1 (0.8%) 95% CI: 0.00–2.37	NS	1 (0.3%) 95% CI: 0.00–0.86	2 (5.3%) 95% CI: 0.00–12.42	<0.05	3 (0.7%) 95% CI: 0.00–1.52
CRF02_AG	3 (1.1%) 95% CI: 0.00–2.33	8 (6.5%) 95% CI: 2.14–10.86	<0.01	2 (0.6%) 95% CI: 0.00–1.39	9 (23.7%) 95% CI: 10.18–37.22	<0.01	11 (2.7%) 95% CI: 1.11–4.29
CRF12_BF	3 (1.1%) 95% CI: 0.00–2.33	1 (0.8%) 95% CI: 0.00–2.37	NS	1 (0.3%) 95% CI: 0.00–0.86	3 (7.9) 95% CI: 0.00–16.48	<0.01	4 (1.0%) 95% CI: 0.03–1.97
Total	278	123		363	38		401

NS, not significant; CI, confidence interval.

TABLE 2. HIV-1 subtypes in patients, by age

	Age classes (years)				
HIV-1 subtypes	≤30	31–40	41–50	>50	p ≤50 vs >50
B	44 (88.0%) 95% CI: 78.99–97.01	147 (88.0%) 95% CI: 83.07–92.93	117 (92.1%) 95% CI: 87.41–96.79	44 (77.2%) 95% CI: 66.31–88.09	<0.05
Non-B	6 (12.0%) 95% CI: 2.99–21.01	20 (12.0%) 95% CI: 7.07–16.93	10 (7.9%) 95% CI: 3.21–12.59	13 (22.8%) 95% CI: 11.91–33.69	
Total	50	167	127	57	

CI, confidence interval.

TABLE 3. HIV-1 subtypes in patients, by transmission route

	HIV-1 positive patients				
HIV-1 subtypes	Ex-IVDUs	Heterosexuals	MSM/bisexuals	Others[a]	Total
B	122 (98.4%) 95% CI: 96.19–100.0	155 (79.5%) 95% CI: 73.83–85.17	61 (92.4%) 95% CI: 86.01–98.79	4 (80.0%) 95% CI: 44.94–100.0	342 (87.7%) 95% CI: 84.44–90.96
Non B	2 (1.6%) 95% CI: 0.00–3.81	40 (20.5%) 95% CI: 14.83–26.17	5 (7.6%) 95% CI: 1.21–13.99	1 (20.0%) 95% CI: 0.00–55.06	48 (12.3%) 95% CI: 9.04–15.56
Total	124	195	66	5	390

CI, confidence interval.
[a]Three vertical, one transfusional, one through tattooing.

period of HIV infection. Table 4 shows the prevalence of the subtypes by period of infection, stratified by risk factor. The differences between the various periods were not statistically significant except in the case of heterosexuals between the periods 1993 to 2000 and after 2000. All of the subjects with a non-B subtype who were infected before 1993 were Italian (two men and one woman).

The probable geographical origin of the infection was available in the case of 227 Italian patients (202 B and 25 non-B subtypes) infected as a result of sexual contacts (168 heterosexual, five bisexual and 54 MSM). Two hundred and seven patients (193 B and 14 non-B subtypes) were infected in Italy and 20 (nine B and 11 non-B subtypes) infected abroad or after sexual contacts in Italy with partners from abroad. The difference between the prevalence of non-B subtypes among the patients who contracted the infection in Italy (6.8%; 95% CI, 3.37–10.23) and those who contracted it abroad or with foreign partners (55.0%; 95% CI, 33.20–76.80) was statistically significant (p <0.01). The difference is statistically significant (p <0.01) in the case of heterosexuals

TABLE 4. HIV-1 subtypes by periods in which HIV-1 infection presumably occurred and transmission route

	Periods in which HIV-1 infection presumably occurred (years)									
	<1993		1993–2000		>2000		Total			
	Subtypes		Subtypes		Subtypes		Subtypes			
HIV-1 positive patients	B	Non- B	B	Non-B	B	Non-B	B	Non-B		
Ex-IVDUs	67 (98.5%)	1 (1.5%)	41 (100%)	0 (0%)	13 (100%)	0 (0%)	121 (99.2%)	1 (0.8%)		
	95% CI: 95.61–100.0	95% CI: 0.00–4.39	95% CI: 100.0–100.0	95% CI: 0.00–0.00	95% CI: 100.0–100.0	95% CI: 0.00–0.00	95% CI: 97.62–100.0	95% CI: 0.00–2.38		
Heterosexuals	24 (92.3%)	2 (7.7%)	54 (88.5%)	7 (11.5%)	71 (69.6%)	31 (30.4%)	149 (78.8%)	40 (21.2%)		
	95% CI: 82.05–100.0	95% CI: 0.00–17.95	95% CI: 80.49–96.51	95% CI: 3.49–19.51	95% CI: 60.67–78.53	95% CI: 21.47–39.33	95% CI: 72.97–84.63	95% CI: 15.37–27.03		
MSM/bisexuals	8 (100%)	0 (0%)	20 (90.9%)	2 (9.1%)	33 (91.7%)	3 (8.3%)	61 (92.4%)	5 (7.6%)		
	95% CI: 100.0–100.0	95% CI: 0.00–0.00	95% CI: 78.88–100.0	95% CI: 0.00–21.12	95% CI: 82.69–100.0	95% CI: 0.00–17.31	95% CI: 86.01–98.79	95% CI: 1.21–13.99		
Others[a]	3 (100%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	4 (80.0%)	1 (20.0%)		
	95% CI: 100.0–100.0	95% CI: 0.00–0.00	95% CI: 100.0–100.0	95% CI: 0.00–0.00	95% CI: 0.00–0.00	95% CI: 100.0–100.0	95% CI: 44.94–100.0	95% CI: 0.00–55.06		
Total	102 (97.1%)	3 (2.9%)	116 (92.8%)	9 (7.2%)	117 (77.0%)	35 (23.0%)	335 (87.7%)	47 (12.3%)		
	95% CI: 93.89–100.0	95% CI: 0.00–6.11	95% CI: 88.27–97.33	95% CI: 2.67–11.73	95% CI: 70.31–83.69	95% CI: 16.31–29.69	95% CI: 84.41–90.99	95% CI: 9.01–15.59		

CI, confidence interval.
[a]Three vertical, one transfusional, one through tattooing.

(6.7%; 95% CI, 2.69–10.71 vs 57.9%; 95% CI, 35.70–80.10) but not in the case of MSM/bisexuals (6.9%; 95% CI, 0.38–13.42 vs 0%; 95% CI, 0.00–0.00).

Discussion

HIV-1 infection in Italy spread in the early 1980s, especially among IVDUs and MSM. As in the USA and other countries of Western Europe, the B subtype predominated. The number of heterosexuals infected has gradually increased over time: data from the Italian Ministry of Health shows that the percentage of AIDS cases among heterosexuals rose from 14.8% before 1997 to 44.7% in 2007–2008 (among MSM, the increase was from 15.5% to 22.9%) [25]. The proportion of foreigners with a new diagnoses of HIV infection increased from 11% in 1992 to 32.0% in 2007 and, among them, heterosexual contacts represent the most frequent form of transmission [25]. During this period, Italy experienced a substantial increase in immigration, especially from countries outside the European Community. The flow of immigrants in 2008 was estimated to be approximately 350 000 people, leading to an approximately 6% prevalence of foreign citizens resident in Italy [26].

The immigration of people from countries with a high prevalence of non-B subtypes has led to the circulation of new subtypes in Italy, as in the rest of Europe [12,13,27]. A total of 12.2% of the patients in this study were infected with a non-B subtype, particularly F1 and CRFs (which account for about one-third of all non-B subtypes). This is in line with the findings of studies carried out elsewhere in Italy, where the observed prevalence ranges from 4% to 12.6% [14,28,29], but lower than in other European countries where the prevalence of non-B subtypes is as high as 20–40% [16,30]. The prevalence of non-B subtypes among the Italian patients included in this study was much lower (7.7%) than among non-Italians (55.3%), who therefore represent a considerable reservoir for the sexual transmission of non-B subtypes. Heterosexual contacts were the most important route of transmission, accounting for all of the infections in non-Italians; among the Italians, the prevalence of non-B subtypes was higher among heterosexuals than MSM and ex-IVDUs (mainly among heterosexuals infected abroad or in Italy by partners from abroad). Furthermore, the prevalence of non-B subtypes among heterosexuals increased from 2.9% before 1993 to 23.0% after 2000.

One limitation of the findings of this study is that the absence of seroconversion data (except in very few cases) means that the first positive HIV antibody test was used as a surrogate for the date of infection, which may actually have

occurred much earlier in some cases (which means the entry of non-B subtypes into Italy may also have been earlier). However, as the same approach was used for all of the study periods, the trend is plausible and consistent with the findings of other studies [14].

The fact that the percentage of non-B subtypes among MSM has not changed over time may indicate a degree of stability in their sexual behaviour. Among the ex-IVDUs, non-B subtypes were only observed in two patients, both of whom were infected before 1993. However, it must be remembered that in Italy the number of new infections among IVDUs decreased from 69.0% to 8.6% of all new cases between 1985 and 2007, whereas the cases attributable to sexual transmission (heterosexual and MSM/bisexual) increased from 13.3% to 73.7% [25]. According to the Italian National Statistics Office, the majority of subjects with HIV infection are no longer young IVDUs but mature adults infected as a result of heterosexual contacts (i.e. the group in which we found the highest percentage of non-B subtypes).

In conclusion, the prevalence of non-B subtypes is increasing in this area of Italy because of the increase in the number of immigrants (including those with new HIV infections) and increased sexual transmission between them and Italians. Given this increase in the prevalence of non-B subtypes, greater efforts should be made to explore their biological characteristics and clinical implications, particularly as a greater number of older people will be affected.

Transparency Declaration

All authors have no conflict of interest to declare.

References

1. Robertson DL, Anderson JP, Bradac JA *et al.* HIV-1 nomenclature proposal. *Science* 2000; 288: 55–56.
2. Korber B, Gaschen B, Yusim K, Thakallapally R, Kesmir C, Detours V. Evolutionary and immunological implications of contemporary HIV-1 variation. *Br Med Bull* 2001; 58: 19–42.
3. Peeters M. Recombinant HIV sequences: their role in the global epidemic. In: Kuiken C, Foley B, Hahn B, McCutchan FE, Mellors JW, Mullins JI, Sodroski J, Wolinsky S, Korber B, eds, *HIV sequence compendium 2000*. Los Alamos: Los Alamos National Laboratory, 2001; 54–72.
4. Hemelaar J, Gouws E, Ghys PD, Osmanov S. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. *AIDS* 2006; 20: 13–23.
5. Osmanov S, Pattou C, Walker N, Schwardländer B, Esparza J; WHO-UNAIDS Network for HIV Isolation and Characterization. Estimated global distribution and regional spread of HIV-1 genetic subtypes in the year 2000. *J Acquir Immune Defic Syndr* 2002; 29: 184–190.
6. McCutchan FE. Understanding the genetic diversity of HIV-1. *AIDS* 2000; 14 (suppl): 31–44.
7. Gao F, Robertson DL, Morrison SG *et al.* The heterosexual human immunodeficiency virus type 1 epidemic in Thailand is caused by an intersubtype (A/E) recombinant of African origin. *J Virol* 1996; 70: 7013–7029.
8. Van Harmelen J, Wood R, Lambrick M, Rybicki EP, Williamson AL, Williamson C. An association between HIV-1 subtypes and mode of transmission in Cape Town, South Africa. *AIDS* 1997; 11: 81–87.
9. McCutchan FE, Hegerich PA, Brennan TP *et al.* Genetic variants of HIV-1 in Thailand. *AIDS Res Hum Retroviruses* 1992; 8: 1887–1895.
10. Subbarao S, Vanichseni S, Hu DJ *et al.* Genetic characterization of incident HIV type 1 subtype E and B strains from a prospective cohort of injecting drug users in Bangkok, Thailand. *AIDS Res Hum Retroviruses* 2000; 16: 699–707.
11. Irwin KL, Pau CP, Lupo D *et al.* Presence of human immunodeficiency virus (HIV) type 1 subtype A infection in a New York community with high HIV prevalence: a sentinel site for monitoring HIV genetic diversity in North America. Centers for Disease Control and Prevention-Bronx Lebanon HIV Serosurvay Team. *J Infect Dis* 1997; 176: 1629–1633.
12. Böni J, Pyra H, Gebhardt M *et al.* High frequency of non-B subtypes in newly diagnosed HIV-1 infections in Switzerland. *J Acquir Immune Defic Syndr* 1999; 22: 174–179.
13. Couturier E, Damond F, Roques P *et al.* HIV-1 diversity in France, 1996–1998. The AC 11 laboratory network. *AIDS* 2000; 14: 289–296.
14. Balotta C, Facchi G, Violin M *et al.* ICONA Study Group Increasing prevalence of non-clade B HIV-1 strains in heterosexual men and women, as monitored by analysis of reverse transcriptase and protease sequences. *J Acquir Immune Defic Syndr* 2001; 27: 499–505.
15. Holguin A, Aracil B, Alvarez A, Barros C, Soriano V. Prevalence of human immunodeficiency virus type 1 (HIV-1) non-B subtypes in foreigners living in Madrid, Spain, and comparison of the performances of the AMPICOR HIV-1 MONITOR version 1.0 and the new automated version 1.5. *J Clin Microbiol* 2001; 39: 1850–1854.
16. Deroo S, Robert I, Fontaine E *et al.* HIV-1 subtypes in Luxembourg, 1983–2000. *AIDS* 2002; 16: 2461–2467.
17. Kanki PJ, Hamel DJ, Sankalé JL *et al.* Human immunodeficiency virus type 1 subtypes differ in disease progression. *J Infect Dis* 1999; 179: 68–73.
18. Kaleebu P, French N, Mahe C *et al.* Effect of human immunodeficiency virus (HIV) type 1 envelope subtype A and D on disease progression in a large cohort of HIV-1 positive persons in Uganda. *J Infect Dis* 2002; 185: 1244–1250.
19. Alaeus A, Lidman K, Björkman A, Giesecke J, Albert J. Similar rate of disease progression among individuals infected with HIV-1 genetic subtypes A–D. *AIDS* 1999; 13: 901–907.
20. Tscherning C, Alaeus A, Fredriksson R *et al.* Differences in chemokine coreceptor usage between genetic subtypes of HIV-1. *Virology* 1998; 241: 181–188.
21. Palmer S, Alaeus A, Albert J, Cox S. Drug susceptibility of subtypes A,B,C,D. and E human immunodeficiency virus type 1 primary isolates. *AIDS Res Hum Retroviruses* 1998; 14: 157–162.
22. Hirsch MS, Conway B, D'Aquila RT *et al.* Antiretroviral drug resistance testing in adults with HIV infection: implications for clinical management. International AIDS Society-USA Panel. *JAMA* 1998; 279: 1984–1991.
23. Loemba H, Brenner B, Parniak MA *et al.* Genetic divergence of human immunodeficiency virus type 1 Ethiopian clade C reverse transcriptase (RT) and rapid development of resistance against non-nucleoside inhibitors of RT. *Antimicrob Agents Chemother* 2002; 46: 2087–2094.

24. Descamps D, Apetrei C, Collin G, Damond F, Simon F, Brun-Vezinet F. Naturally occurring decreased susceptibility of HIV-1 subtype G to protease inhibitors. *AIDS* 1998; 12: 1109–1111.
25. Suligoi B, Boros S, Camoni L, Lepore D. Notiziario dell'Istituto Superiore di Sanità. 2009; 22, No. 3, (suppl 1): ISSN0394–9303.
26. Caritas-Migrantes Foundation. *Immigrazione. Dossier statistico 2008. XVIII rapporto*. Rome: Edizioni Idos, 2008.
27. Devereux H, Loveday C, Burke A, Dann L, Johnson M, Phillips A. The prevalence of non-B subtype HIV-1 in a London HIV/AIDS outpatient clinic. *AIDS* 1999; 13: 142.
28. Monno L, Brindicci G, Lo Caputo S *et al.* HIV-1 subtypes and circulating recombinant forms (CRFs) from HIV-infected patients residing in two regions of central and southern Italy. *J Med Virol* 2005; 75: 483–490.
29. Tramuto F, Vitale F, Bonura F, Romano N. Group for HIV-1 Antiretroviral Studies in Sicily. Detection of HIV type 1 non-B subtypes in Sicily, Italy. *AIDS Res Hum Retroviruses* 2004; 20: 251–254.
30. Snoeck J, Van Dooren S, Van Laethem K *et al.* Prevalence and origin of HIV-1 group M subtypes among patients attending a Belgian hospital in 1999. *Virus Res* 2002; 85: 95–107.